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(54) Title: SECRETED PROTEINS

(57) Abstract

Novel proteins are disclosed.

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# SECRETED PROTEINS

This application is a continuation-in-part of application Ser. No. 08/634,325, filed April 18, 1996.

## FIELD OF THE INVENTION

The present invention provides novel proteins , along with therapeutic, diagnostic and research utilities for these proteins.

## BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these proteins that the present invention is directed.

## SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 6 to nucleotide 545;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK296 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK296 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 6 to nucleotide 545; the nucleotide sequence of the full-length protein coding sequence of clone AK296 deposited under accession number ATCC 98026; or the nucleotide sequence of the mature protein coding sequence of clone AK296 deposited under accession number ATCC 98026. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026. In yet other preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:2 from amino acid 128 to amino acid 153.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acid 128 to amino acid 153;

(c) fragments of the amino acid sequence of SEQ ID NO:2; and

(d) the amino acid sequence encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:2 or the amino acid sequence of SEQ ID NO:2 from amino acid 128 to amino acid 153.

In one embodiment, the present invention provides a composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 136 to nucleotide 1473;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK533 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK533 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:3 from nucleotide 136 to nucleotide 1473; the nucleotide sequence of the full-length

protein coding sequence of clone AK533 deposited under accession number ATCC 98026; or the nucleotide sequence of the mature protein coding sequence of clone AK533 deposited under accession number ATCC 98026. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026. In yet other preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:4 from amino acid 58 to amino acid 73.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) the amino acid sequence of SEQ ID NO:4 from amino acid 58 to amino acid 73;

(c) fragments of the amino acid sequence of SEQ ID NO:4; and

(d) the amino acid sequence encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:4 or the amino acid sequence of SEQ ID NO:4 from amino acid 58 to amino acid 73.

In one embodiment, the present invention provides a composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 186 to nucleotide 1532;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 261 to nucleotide 1532;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK647 deposited under accession number ATCC 98026;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK647 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above; and

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:5 from nucleotide 186 to nucleotide 1532; the nucleotide sequence of SEQ ID NO:5 from nucleotide 261 to nucleotide 1532; the nucleotide sequence of the full-length protein coding sequence of clone AK647 deposited under accession number ATCC 98026; or the nucleotide sequence of the mature protein coding sequence of clone AK647 deposited under accession number ATCC 98026. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026. In yet other preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 104. In further preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 93.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 104;

(c) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 93;

(d) fragments of the amino acid sequence of SEQ ID NO:6; and

(e) the amino acid sequence encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:6, or the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 104, or the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 93.

In one embodiment, the present invention provides a composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 30 to nucleotide 701;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 99 to nucleotide 701;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AM610 deposited under accession number ATCC 98026;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM610 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above; and

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:9 from nucleotide 30 to nucleotide 701; the nucleotide sequence of SEQ ID NO:9 from

nucleotide 99 to nucleotide 701; the nucleotide sequence of the full-length protein coding sequence of clone AM610 deposited under accession number ATCC 98026; or the nucleotide sequence of the mature protein coding sequence of clone AM610 deposited under accession number ATCC 98026. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026. In yet other preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 96. In further preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 81.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 96;

(c) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 81;

(d) fragments of the amino acid sequence of SEQ ID NO:10; and

(e) the amino acid sequence encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:10, or the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 96, or the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 81.

In one embodiment, the present invention provides a composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 132 to nucleotide 752;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR260 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR260 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:11 from nucleotide 132 to nucleotide 752; the nucleotide sequence of the full-length protein coding sequence of clone AR260 deposited under accession number ATCC 98026; or the nucleotide sequence of the mature protein coding sequence of clone AR260 deposited under accession number ATCC 98026. In other preferred embodiments, the polynucleotide encodes the full-length or mature protein encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026. In yet other preferred embodiments, such polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:12 from amino acid 1 to amino acid 85.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12;

(b) the amino acid sequence of SEQ ID NO:12 from amino acid 1 to amino acid 85;

(c) fragments of the amino acid sequence of SEQ ID NO:12; and

(d) the amino acid sequence encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:12 or the amino acid sequence of SEQ ID NO:12 from amino acid 1 to amino acid 85.

Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

## BRIEF DESCRIPTION OF FIGURES

Fig. 1 is an autoradiograph evidencing the expression of clone AK296 in COS cells (expressed band indicated by a dot).

Fig. 2 is an autoradiograph evidencing the expression of clone AK533 in COS cells (expressed band(s) indicated by dot(s)).

Fig. 3 is an autoradiograph evidencing the expression of clone AK647 in COS cells.

Fig. 4 is an autoradiograph evidencing the expression of clone AM610 in COS cells (expressed band(s) indicated by dot(s)).

Fig. 5 is an autoradiograph evidencing the expression of clone AR260 in COS cells (expressed band indicated by a dot).

## DETAILED DESCRIPTION

### ISOLATED PROTEINS

Nucleotide and amino acid sequences, as presently determined, are reported below for each clone and protein disclosed in the present application. The nucleotide sequence of each clone can readily be determined by sequencing of the deposited clone in accordance with known methods. The predicted amino acid sequence (both full-length and mature) can then be determined from such nucleotide sequence. The amino acid sequence of the protein encoded by a particular clone can also be determined by expression of the clone in a suitable host cell, collecting the protein and determining its sequence. For each disclosed protein applicants have identified what they have

determined to be the reading frame best identifiable with sequence information available at the time of filing.

As used herein a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g. , receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

Protein "AK296"

One protein of the present invention has been identified as protein "AK296". A partial cDNA clone encoding AK296 was first isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins. The nucleotide sequence of such partial cDNA was determined and searched against the GenBank and GeneSeq databases using BLASTN/BLASTX and FASTA search protocols. The search revealed at least some identity with sequences identified as T75226 (yc86g12.r1 Homo sapiens cDNA clone 22958 5'), AA171668 (zo94g03.r1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone 594580 5'), T22009 (Human gene signature HUMGS03552), and H80457 (yu75a07.r1 Homo sapiens cDNA clone 239604 5'). The human cDNA clone corresponding to the EST database entry was ordered from Genome Systems, Inc., St. Louis, Mo, a distributor of the I.M.A.G.E. Consortium library. The clone received from the distributor was examined and determined to be a full-length clone, including a 5' end and 3' UTR (including a polyA tail). This full-length clone is also referred to herein as "AK296".

Applicants' methods identified clone AK296 as encoding a secreted protein.

The nucleotide sequence of AK296 as presently determined is reported in SEQ ID NO:1. What applicants believe is the proper reading frame and the predicted amino acid sequence of the AK296 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:2.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AK296 should be approximately 1264 bp.

Protein "AK533"

One protein of the present invention has been identified as protein "AK533". A partial cDNA clone encoding AK533 was first isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins. The nucleotide sequence of such partial cDNA was determined and searched against the GenBank and GeneSeq databases using BLASTN/BLASTX and FASTA search protocols. The search revealed at least some identity with sequences identified as T55939 (yb82h07.r1 Homo sapiens cDNA clone 77725 5'), U63008 (Human homogentisate dioxygenase (HGO) mRNA, complete cds), AA137764 (mq28c07.r1 Barstead MPLRB1 Mus musculus cDNA clone 580044 5' similar to TR G984327 G984327 2,5 DIHYDROXYPHENYLACETATE OXIDASE), and Z75048 (H.sapiens mRNA for homogentisate 1,2-dioxygenase). The predicted amino acid sequence disclosed herein for AK533 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AK533 protein demonstrated at least some identity with sequences identified as U63008 (homogentisate dioxygenase [Homo sapiens]) and U30797 (ENU30797_1 2,5 dihydroxyphenylacetate oxidase [Emericella nidulans]). The TopPredII computer program predicts two potential transmembrane domains within the AK533 protein sequence, one around amino acid 350 and another around amino acid 400 of SEQ ID NO:4. The human cDNA clone corresponding to the EST database entry was ordered from Genome Systems, Inc., St. Louis, Mo, a distributor of the I.M.A.G.E. Consortium library. The clone received from the distributor was examined and determined to be a full-length clone, including a 5' end and 3' UTR (including a polyA tail). This full-length clone is also referred to herein as "AK533".

Applicants' methods identified clone AK533 as encoding a secreted protein.

The nucleotide sequence of AK533 as presently determined is reported in SEQ ID NO:3. What applicants believe is the proper reading frame and the predicted amino acid sequence of the AK533 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:4.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AK533 should be approximately 1751 bp.

Protein "AK647"

One protein of the present invention has been identified as protein "AK647". A partial cDNA clone encoding AK647 was first isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins. The nucleotide sequence of such partial cDNA was determined and searched against the GenBank and GeneSeq databases using BLASTN/BLASTX and FASTA search protocols. The search revealed at least some identity with

an EST identified as H17726 (ym40a05.r1 Homo sapiens cDNA clone 50483 5'), U03877 (Human extracellular protein (S1-5) mRNA, complete cds), N50529 (yy89c07.s1 Homo sapiens cDNA clone 280716 3'), and T21312 (Human gene signature HUMGS02672). The predicted amino acid sequence disclosed herein for AK647 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AK647 protein demonstrated at least some identity with sequences identified as U13646 (homeotic region most like HMPB_DROME homeotic proboscipedia protein [Caenorhabditis elegans]), U03877 (extracellular protein [Homo sapiens]), R11150 (Fibulin C), and U03272 (HSU03272_1 fibrillin-2 [Homo sapiens]). The human cDNA clone corresponding to the EST database entry was ordered from Genome Systems, Inc., St. Louis, Mo, a distributor of the I.M.A.G.E. Consortium library. The clone received from the distributor was examined and determined to be a full-length clone, including a 5' end and 3' UTR (including a polyA tail). This full-length clone is also referred to herein as "AK647".

Applicants' methods identified clone AK647 as encoding a secreted protein.

The nucleotide sequence of the 5' portion of AK647 as presently determined is reported in SEQ ID NO:5. What applicants believe is the proper reading frame and the predicted amino acid sequence of the AK647 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6. Amino acids 13 to 25 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 26, or are a transmembrane domain. Additional nucleotide sequence from the 3' portion of AK647, including the polyA tail, is reported in SEQ ID NO:7.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AK647 should be approximately 2383 bp.

Protein "AM610"

One protein of the present invention has been identified as protein "AM610". A partial cDNA clone encoding AM610 was first isolated from a human fetal kidney cDNA library using methods which are selective for cDNAs encoding secreted proteins. The nucleotide sequence of such partial cDNA was determined and searched against the GenBank and GeneSeq databases using BLASTN/BLASTX and FASTA search protocols. The search revealed at least some identity with an EST identified as H09926 (ym01a10.s1 Homo sapiens cDNA clone 46249 3'), X98993 (R.norvegicus mRNA for microvascular endothelial differentiation gene 1), AA129494 (zl11d12.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 501623 3'), AA045792 (zk68h09.r1 Soares pregnant uterus

NbHPU Homo sapiens cDNA clone 488033 5'), H09925 (ym01a10.r1 Homo sapiens cDNA clone 46249 5' similar to SP:DNAJ_ERYRH Q05646 DNAJ), and R14298 (yf80c02.r1 Homo sapiens cDNA). The predicted amino acid sequence disclosed herein for AM610 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted AM610 protein demonstrated at least some identity with sequences identified as X98993 (putative microvascular endothelial differentiation gene 1 [Rattus norvegicus]), R90680 (Mouse cysteine string protein), and L08110 (ERYDNAKJ_2 dnaJ gene product [Erysipelothrix rhusiopathiae]). The human cDNA clone corresponding to the EST database entry was ordered from Genome Systems, Inc., St. Louis, Mo, a distributor of the I.M.A.G.E. Consortium library. The clone received from the distributor was examined and determined to be a full-length clone, including a 5' end and 3' UTR (including a polyA tail). This full-length clone is also referred to herein as "AM610".

Applicants' methods identified clone AM610 as encoding a secreted protein.

The partial nucleotide sequence of AM610, including its 3' end and any identified polyA tail, as presently determined is reported in SEQ ID NO:9. What applicants believe is the proper reading frame and the predicted amino acid sequence of the AM610 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:10. Amino acids 11 to 23 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 24, or are a transmembrane domain. Additional nucleotide sequence from the 5' portion of AM610 is reported in SEQ ID NO:8.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AM610 should be approximately 1900 bp.

Protein "AR260"

One protein of the present invention has been identified as protein "AR260". A partial cDNA clone encoding AR260 was first isolated from a human adult retina cDNA library using methods which are selective for cDNAs encoding secreted proteins. The nucleotide sequence of such partial cDNA was determined and searched against the GenBank and GeneSeq databases using BLASTN/BLASTX and FASTA search protocols. The search revealed at least some identity with an EST identified as R52804 (yg99g12.r1 Homo sapiens cDNA clone 41757 5'), D55856 (Human fetal brain cDNA 5'-end GEN-404C03), AA253126 (zr52e09.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 667048 5'), and R96245 (yq36a02.r1 Homo sapiens cDNA clone 197834 5'). The human

cDNA clone corresponding to the EST database entry was ordered from Genome Systems, Inc., St. Louis, Mo, a distributor of the I.M.A.G.E. Consortium library. The clone received from the distributor was examined and determined to be a full-length clone, including a 5' end and 3' UTR (including a polyA tail). This full-length clone is also referred to herein as "AR260".

Applicants' methods identified clone AR260 as encoding a secreted protein.

The nucleotide sequence of AR260 as presently determined is reported in SEQ ID NO:11. What applicants believe is the proper reading frame and the predicted amino acid sequence of the AR260 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:12.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone AR260 should be approximately 1900 bp.

Deposit of Clones

Clones AK296, AK533, AK647, AM610 and AR260 were deposited on April 17, 1996 with the American Type Culture Collection under accession number ATCC 98026, from which each clone comprising a particular polynucleotide is obtainable. Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit.

Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI) to produce the appropriate fragment for such clone. Each clone was deposited in either the pED6 or pNotS vector depicted in Fig. 1. In some instances, the deposited clone can become "flipped" (i.e., in the reverse orientation) in the deposited isolate. In such instances, the cDNA insert can still be isolated by digestion with EcoRI and NotI. However, NotI will then produce the 5' site and EcoRI will produce the 3' site for placement of the cDNA in proper orientation for expression in a suitable vector. The cDNA may also be expressed from the vectors in which they were deposited.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences.

In the sequences listed above which include an N at position 2, that position is occupied in preferred probes/primers by a biotinylated phosphoaramidite residue rather

than a nucleotide (such as , for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramadite) (Glen Research, cat. no. 10-1953)).

The design of the oligonucleotide probe should preferably follow these parameters:

(a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;

(b) It should be designed to have a $T_m$ of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labeled with g-$^{32}$P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4e+6 dpm/pmole.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml. The culture should preferably be grown to saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1e+6 dpm/mL. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed

by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein may also be determinable from the amino acid sequence of the full-length form.

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed proteins; that is, naturally-occurring alternative forms of the isolated proteins which are identical, homologous or related to that encoded by the polynucleotides disclosed herein.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The isolated polynucleotide endcoing the protein of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell

strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin

(TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art

(see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

## USES AND BIOLOGICAL ACTIVITY

The proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

### Research Uses and Utilities

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular

Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology

133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack

of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, *89*:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected

cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I $\alpha$ chain protein and $\beta_2$ microglobulin protein or an MHC class II $\alpha$ chain protein and an MHC class II $\beta$ chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj

et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or

*ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also

include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium ).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes,

fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting

therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Cadherin/Tumor Invasion Suppressor Activity

Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to tumor growth and metastasis. Cadherin malfunction is also implicated in other human diseases, such as pemphigus vulgaris and pemphigus foliaceus (auto-immune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the first cadherin domain provide the basis for homophilic adhesion; modification of this recognition site can change the specificity of a cadherin so that instead of recognizing only itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherins.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells become invasive and the cancer metastasizes. Transfection of cancer cell lines with

polynucleotides expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells' adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth. Thus, reintroducing E-cadherin expression reverts carcinomas to a less advanced stage. It is likely that other cadherins have the same invasion suppressor role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or polynucleotides into cancer cells can reduce or eliminate the cancerous changes observed in these cells by providing normal cadherin expression.

Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be substituted in these cells for the inappropriately expressed cadherins, restoring normal cell adhesive properties and reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block the adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be detected by the use of a cadherin-binding antibody.

Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and polynucleotides of the present invention encoding such protein fragments, can also be used to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects. Additionally, fragments of proteins of the present invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynucleotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

Assays for cadherin adhesive and invasive suppressor activity include, without limitation, those described in: Hortsch et al. J Biol Chem 270 (32): 18809-18817, 1995; Miyaki et al. Oncogene 11: 2547-2552, 1995; Ozawa et al. Cell 63: 1033-1038, 1990.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen

in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

## ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following

presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunolgobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other

hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The

pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1ng to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting

and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns.

In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorbtion of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth

and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Jacobs, Kenneth
McCoy, John M.
Racie, Lisa A.
LaVallie, Edward R.
Merberg, David
Treacy, Maurice
Evans, Cheryl
Agostino, Michael

(ii) TITLE OF INVENTION: SECRETED PROTEINS

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sprunger, Suzanne A.
(B) REGISTRATION NUMBER: P-41,323

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(A) TELEPHONE: (617) 498-8284
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1230 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGACCATGAT TACGCCAAGC TTGGCACGAG GCAGGGAGGT CCTGACCCCA ACGAGCACTT 60

CTGACAATGA GACCAGAGAC TCCTCAATTA TTGATCCAGG AACTGAGCAA GATCTTCCTT 120

CCCCTGAAAA TAGTTCTGTT AAAGAATACC GAATGGAAGT TCCATCTTCG TTTTCAGAAG 180

ACATGTCAAA TATCAGGTCA CAGCATGCAG AAGAACAGTC CAACAATGGT AGATATGACG 240

ATTGTAAAGA ATTTAAAGAC CTCCACTGTT CCAAGGATTC TACCCTAGCC GAGGAAGAAT 300

CTGAGTTCCC TTCTACTTCT ATCTCTGCAG TTCTGTCTGA CTTAGCTGAC TTGAGAAGCT 360

GTGATGGCCA AGCTTTGCCC TCCCAGGACC CTGAGGTTGC TTTATCTCTC AGTTGTGGCC 420

ATTCCAGAGG ACTCTTTAGT CATATGCAGC AACATGACAT TTTAGATACC CTGTGTAGGA 480

CCATTGAATC TACAATCCAT GTCGTCACAA GGATATCTGG CAAAGGAAAC CAAGCTGCTT 540

CTTGACATTA GGTGTAGCAT GTCTACTTTT AAGTCCCTCA CCCCCAACCC CCATGCTGTT 600

TGTATAAGTT TTGCTTATTT GTTTTTGTGC TTCAGTTTGT CCAGTGCTCT CTGCTTGAAT 660

GGCAAGATAG ATTTATAGGC TTAATTCTTG GTCAGGCAGA ACTCCAGATG AAAAAAACTT 720

GCATCTTCAG TATACTTCCT AAAGGGCAAT CAGATAATGG ATATGTTTTA TGTAATTAAG 780

AGTTCACTTT AGTGGCTTTC ATTTAATATG GCTGTCTGGG AAGAACAGGG TTGCCTAGCC 840

CTGTACAATG TAATTTAAAC TTACAGCATT TTTACTGTGT ATGATATGGT GTCCTCTGTG 900

CCAGTTTTGT ACCTTATAGA GGCAGATTGC CTCCGATCGC TGTGGTTATT ATTATCAAAA 960

TTAAGTTTAC TTGTATACGG AACAACCACA AGAAATTTGA TTCTGTAAAG AATCCTCTTT 1020

AGCTGTGGCC TGGCAGTATA TAAATGGTGC TTTATTTAAC AGAATACCTG TGGAGGAAAT 1080

AAAGCACACT TGATGTAAAA ATAATTGTTT TATTTTTATT GACATGACTG ATTGATTGCT 1140

ATTCTGTGCA CTTAATTAAA CTGATTGTGA TGACTTTTAA AAAAAAAAAA AAAAAAAAAA 1200

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1230

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ile Thr Pro Ser Leu Ala Arg Gly Arg Glu Val Leu Thr Pro Thr
1               5                  10                  15

Ser Thr Ser Asp Asn Glu Thr Arg Asp Ser Ser Ile Ile Asp Pro Gly
            20                  25                  30

Thr Glu Gln Asp Leu Pro Ser Pro Glu Asn Ser Ser Val Lys Glu Tyr
        35                  40                  45

Arg Met Glu Val Pro Ser Ser Phe Ser Glu Asp Met Ser Asn Ile Arg
    50                  55                  60

Ser Gln His Ala Glu Glu Gln Ser Asn Asn Gly Arg Tyr Asp Asp Cys
65                  70                  75                  80

Lys Glu Phe Lys Asp Leu His Cys Ser Lys Asp Ser Thr Leu Ala Glu
                85                  90                  95

Glu Glu Ser Glu Phe Pro Ser Thr Ser Ile Ser Ala Val Leu Ser Asp
            100                 105                 110

Leu Ala Asp Leu Arg Ser Cys Asp Gly Gln Ala Leu Pro Ser Gln Asp
        115                 120                 125

Pro Glu Val Ala Leu Ser Leu Ser Cys Gly His Ser Arg Gly Leu Phe
    130                 135                 140

Ser His Met Gln Gln His Asp Ile Leu Asp Thr Leu Cys Arg Thr Ile
145                 150                 155                 160

Glu Ser Thr Ile His Val Val Thr Arg Ile Ser Gly Lys Gly Asn Gln
                165                 170                 175

Ala Ala Ser
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1721 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CACGAGCAGT GAAGCGCAGT GAAGCAGTGG GAACCGGAAT ATCCAAAGAG TGGTTTGAAG 60

GAGAAAGAAG CATTGTGGCT TTATATCCTC TGGGCCTGGG TTTCCTGAAG TCACCACACA 120

TAGAGGAGAG AGAAAATGGC TGAGTTAAAG TACATTTCTG GATTTGGGAA TGAGTGTTCT 180

TCAGAGGATC CTCGCTGCCC AGGTTCCCTG CCAGAAGGAC AGAATAATCC TCAGGTCTGC 240

CCCTACAATC TCTATGCTGA GCAGCTCTCA GGATCGGCTT TCACTTGTCC ACGGAGCACC 300

AATAAGAGAA GCTGGCTGTA TAGGATTCTA CCTTCAGTTT CTCACAAGCC CTTTGAATCC 360

ATTGACGAAG GCCATGTCAC TCACAACTGG GATGAAGTTG ATCCTGATCC TAACCAGCTT 420

AGATGGAAAC CATTTGAGAT TCCAAAAGCA TCTCAGAAGA AAGTAGACTT TGTGAGTGGC 480

CTGCATACCT TGTGTGGAGC TGGAGACATA AAGTCTAACA ATGGGCTTGC TATCCACATT 540

TTCCTCTGCA ATACCTCCAT GGAGAACAGA TGCTTTTACA ATTCAGATGG GGACTTCTTG 600

ATTGTTCCGC AGAAAGGGAA CCTTCTCATT TACACCGAGT TTGGCAAGAT GCTTGTACAG 660

CCCAATGAGA TCTGCGTCAT TCAGAGAGGA ATGCGGTTCA GCATAGATGT CTTTGAGGAG 720

ACCAGGGGCT ACATCTTGGA GGTCTATGGT GTCCACTTTG AGTTACCTGA CCTTGGACCA 780

ATTGGGGCCA ATGGCTTGGC CAATCCTCGT GATTTCTTGA TACCCATTGC CTGGTATGAG 840

GATCGCCAAG TACCAGGTGG TTACACGGTC ATTAATAAAT ACCAGGGCAA GCTGTTTGCT 900

GCCAAACAGG ATGTCTCCCC GTTCAATGTT GTGGCCTGGC ACGGGAATTA TACACCCTAC 960

AAGTACAACC TGAAGAATTT CATGGTTATC AACTCAGTGG CCTTTGACCA TGCAGACCCA 1020

TCCATTTTCA CAGTATTGAC TGCTAAGTCT GTCCGCCCTG GAGTGGCCAT TGCTGATTTT 1080

GTCATCTTCC CACCTCGATG GGGGGTTGCT GATAAGACCT TCAGGCCTCC TTATTACCAT 1140

AGGAACTGCA TGAGTGAGTT CATGGGACTC ATCCGAGGTC ACTATGAGGC AAAGCAAGGT 1200

GGGTTCCTGC CAGGGGGAGG GAGTCTACAC AGCACAATGA CCCCCCATGG ACCTGATGCT 1260

GACTGCTTTG AGAAGGCCAG CAAGGTCAAG CTGGCACCTG AGAGGATTGC CGATGGCACC 1320

ATGGCATTTA TGTTTGAATC ATCTTTAAGT CTGGCGGTCA CAAAGTGGGG ACTCAAGGCC 1380

TCCAGGTGTT TGGATGAGAA CTACCACAAG TGCTGGGAGC CACTCAAGAG CCACTTCACT 1440

CCCAACTCCA GGAACCCAGC AGAACCTAAT TGAGACTGGA ACATTGCTAC CATAATTAAG 1500

AGTAGATTTG TGAAGATTTC TTCAGAATCT CATGCTTTCT GGTAGTATTG GAGGAGGGGG 1560

TTGGTTAAAA TGAAAATTCA CTTTTCATAG TCAAGTAACT CAGAACTTTT ATGGAAACGC 1620

ATTTGCAAAG TTCTATGGCT GTCACCTTAA TTACTCAATA AACTTGCTGG TGTTCTGTGG 1680

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 1721

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 445 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Glu Leu Lys Tyr Ile Ser Gly Phe Gly Asn Glu Cys Ser Ser
1 5 10 15

Glu Asp Pro Arg Cys Pro Gly Ser Leu Pro Glu Gly Gln Asn Asn Pro
20 25 30

Gln Val Cys Pro Tyr Asn Leu Tyr Ala Glu Gln Leu Ser Gly Ser Ala
35 40 45

Phe Thr Cys Pro Arg Ser Thr Asn Lys Arg Ser Trp Leu Tyr Arg Ile
50 55 60

Leu Pro Ser Val Ser His Lys Pro Phe Glu Ser Ile Asp Glu Gly His
65 70 75 80

Val Thr His Asn Trp Asp Glu Val Asp Pro Asp Pro Asn Gln Leu Arg
85 90 95

Trp Lys Pro Phe Glu Ile Pro Lys Ala Ser Gln Lys Lys Val Asp Phe
100 105 110

Val Ser Gly Leu His Thr Leu Cys Gly Ala Gly Asp Ile Lys Ser Asn
115 120 125

Asn Gly Leu Ala Ile His Ile Phe Leu Cys Asn Thr Ser Met Glu Asn
130 135 140

Arg Cys Phe Tyr Asn Ser Asp Gly Asp Phe Leu Ile Val Pro Gln Lys
145 150 155 160

Gly Asn Leu Leu Ile Tyr Thr Glu Phe Gly Lys Met Leu Val Gln Pro
165 170 175

Asn Glu Ile Cys Val Ile Gln Arg Gly Met Arg Phe Ser Ile Asp Val
180 185 190

Phe Glu Glu Thr Arg Gly Tyr Ile Leu Glu Val Tyr Gly Val His Phe
195 200 205

Glu Leu Pro Asp Leu Gly Pro Ile Gly Ala Asn Gly Leu Ala Asn Pro
210 215 220

Arg Asp Phe Leu Ile Pro Ile Ala Trp Tyr Glu Asp Arg Gln Val Pro
225 230 235 240

Gly Gly Tyr Thr Val Ile Asn Lys Tyr Gln Gly Lys Leu Phe Ala Ala
245 250 255

Lys Gln Asp Val Ser Pro Phe Asn Val Val Ala Trp His Gly Asn Tyr
260 265 270

Thr Pro Tyr Lys Tyr Asn Leu Lys Asn Phe Met Val Ile Asn Ser Val
275 280 285

Ala Phe Asp His Ala Asp Pro Ser Ile Phe Thr Val Leu Thr Ala Lys
290 295 300

Ser Val Arg Pro Gly Val Ala Ile Ala Asp Phe Val Ile Phe Pro Pro
305 310 315 320

Arg Trp Gly Val Ala Asp Lys Thr Phe Arg Pro Pro Tyr Tyr His Arg
325 330 335

Asn Cys Met Ser Glu Phe Met Gly Leu Ile Arg Gly His Tyr Glu Ala
340 345 350

Lys Gln Gly Gly Phe Leu Pro Gly Gly Gly Ser Leu His Ser Thr Met
355 360 365

Thr Pro His Gly Pro Asp Ala Asp Cys Phe Glu Lys Ala Ser Lys Val
370 375 380

Lys Leu Ala Pro Glu Arg Ile Ala Asp Gly Thr Met Ala Phe Met Phe
385 390 395 400

Glu Ser Ser Leu Ser Leu Ala Val Thr Lys Trp Gly Leu Lys Ala Ser
405 410 415

Arg Cys Leu Asp Glu Asn Tyr His Lys Cys Trp Glu Pro Leu Lys Ser
420 425 430

His Phe Thr Pro Asn Ser Arg Asn Pro Ala Glu Pro Asn
435 440 445

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2362 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGCTTGGCA CGAGGGGACC CCGGCGCTCT CCCCGTGTCC TCTCCACGAC TCGCTCGGCC 60

CCTCTGGAAT AAAACACCCG CGAGCCCCGA GGGCCCAGAG GAGGCCGACG TGCCCGAGCT 120

CCTCCGGGGG TCCCGCCCGC GAGCTTTCTT CTCGCCTTCG CATCTCCTCC TCGCGCGTCT 180

TGGACATGCC AGGAATAAAA AGGATACTCA CTGTTACCAT TCTGGCTCTC TGTCTTCCAA 240

GCCCTGGGAA TGCACAGGCA CAGTGCACGA ATGGCTTTGA CCTGGATCGC CAGTCAGGAC 300

AGTGTTTAGA TATTGATGAA TGCCGAACCA TCCCCGAGGC CTGCCGAGGA GACATGATGT 360

GTGTTAACCA AAATGGCGGG TATTTATGCA TTCCCCGGAC AAACCCTGTG TATCGAGGGC 420

CCTACTCGAA CCCCTACTCG ACCCCCTACT CAGGTCCGTA CCCAGCAGCT GCCCCACCAC 480

TCTCAGCTCC AAACTATCCC ACGATCTCCA GGCCTCTTAT ATGCCGCTTT GGATACCAGA 540

TGGATGAAAG CAACCAATGT GTGGATGTGG ACGAGTGTGC AACAGATTCC CACCAGTGCA 600

ACCCCACCCA GATCTGCATC AATACTGAAG GCGGGTACAC CTGCTCCTGC ACCGACGGAT 660

ATTGGCTTCT GGAAGGCCAG TGCTTAGACA TTGATGAATG TCGCTATGGT TACTGCCAGC 720

AGCTCTGTGC GAATGTTCCT GGATCCTATT CTTGTACATG CAACCCTGGT TTTACCCTCA 780

ATGAGGATGG AAGGTCTTGC CAAGATGTGA ACGAGTGTGC CACCGAGAAC CCCTGCGTGC 840

AAACCTGCGT CAACACCTAC GGCTCTTTCA TCTGCCGCTG TGACCCAGGA TATGAACTTG 900

AGGAAGATGG CGTTCATTGC AGTGATATGG ACGAGTGCAG CTTCTCTGAG TTCCTCTGCC 960

AACATGAGTG TGTGAACCAG CCCGGCACAT ACTTCTGCTC CTGCCCTCCA GGCTACATCC 1020

TGCTGGATGA CAACCGAAGC TGCCAAGACA TCAACGAATG TGAGCACAGG AACCACACGT 1080

GCAACCTGCA GCAGACGTGC TACAATTTAC AAGGGGGCTT CAAATGCATC GACCCCATCC 1140

GCTGTGAGGA GCCTTATCTG AGGATCAGTG ATAACCGCTG TATGTGTCCT GCTGAGAACC 1200

CTGGCTGCAG AGACCAGCCC TTTACCATCT TGTACCGGGA CATGGACGTG GTGTCAGGAC 1260

GCTCCGTTCC CGCTGACATC TTCCAAATGC AAGCCACGAC CCGCTACCCT GGGGCCTATT 1320

ACATTTTCCA GATCAAATCT GGGAATGAGG GCAGAGAATT TTACATGCGG CAAACGGGCC 1380

CCATCAGTGC CACCCTGGTG ATGACACGCC CCATCAAAGG GCCCCGGGAA ATCCAGCTGG 1440

ACTTGGAAAT GATCACTGTC AACACTGTCA TCAACTTCAG AGGCAGCTCC GTGATCCGAC 1500

TGCGGATATA TGTGTCGCAG TACCCATTCT GAGCCTCGGG CTGGAGCCTC CGACGCTGCC 1560

TCTCATTGGC ACCAAGGGAC AGGAGAAGAG AGGAAATAAC AGAGAGAATG AGAGCGACAC 1620

AGACGTTAGG CATTTCCTGC TGAACGTTTC CCCGAAGAGT CAGCCCCGAC TTCCTGACTC 1680

TCACCTGTAC TATTGCAGAC CTGTCACCCT GCAGGACTTG CCACCCCCAG TTCCTATGAT 1740

ACAGTTATCA AAAAGTATTA TCATTGCTCC CCTGATAGAA GATTGTTGGT GAATTTTCAA 1800

GGCCTTCAGT TTATTTCCAC TATTTTCAAA GAAAATAGAT TAGGTTTGCG GGGGTCTGAG 1860

TCTATGTTCA AAGACTGTGA ACAGCTTGCT GTCACTTCTT CACCTCTTCC ACTCCTTCTC 1920

TCACTGTGTT ACTGCTTTGC AAAGACCCGG GAGCTGGCGG GGAACCCTGG GAGTAGCTAG 1980

TTTGCTTTTT GCGTACACAG AGAAGGCTAT GTAAACAAAC CACAGCAGGA TCGAAGGGTT 2040

TTTAGAGAAT GTGTTTCAAA ACCATGCCTG GTATTTTCAA CCATAAAAGA AGTTTCAGTT 2100

GTCCTTAAAT TTGTATAACG GTTTAATTCT GTCTTGTTCA TTTTGAGTAT TTTTAAAAAA 2160

TATGTCGTAG AATTCCTTCG AAAGGCCTTC AGACACATGC TATGTTCTGT CTTCCCAAAC 2220

CCAGTCTCCT CTCCATTTTA GCCCAGTGTT TTCTTTGAGG ACCCCTTAAT CTTGCTTTCT 2280

TTAGAATTTT TACCCAATTG GATTGGAATG CAGAGGTCTC CAAACTGATT AAATATTTGA 2340

AGAGAAAAAA AAAAAAAAAA AA 2362

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 448 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Pro Gly Ile Lys Arg Ile Leu Thr Val Thr Ile Leu Ala Leu Cys
1 5 10 15

Leu Pro Ser Pro Gly Asn Ala Gln Ala Gln Cys Thr Asn Gly Phe Asp
20 25 30

Leu Asp Arg Gln Ser Gly Gln Cys Leu Asp Ile Asp Glu Cys Arg Thr
35 40 45

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Ile Pro Glu Ala Cys Arg Gly Asp Met Met Cys Val Asn Gln Asn Gly
    50                  55                  60

Gly Tyr Leu Cys Ile Pro Arg Thr Asn Pro Val Tyr Arg Gly Pro Tyr
65                  70                  75                  80

Ser Asn Pro Tyr Ser Thr Pro Tyr Ser Gly Pro Tyr Pro Ala Ala Ala
                85                  90                  95

Pro Pro Leu Ser Ala Pro Asn Tyr Pro Thr Ile Ser Arg Pro Leu Ile
            100                 105                 110

Cys Arg Phe Gly Tyr Gln Met Asp Glu Ser Asn Gln Cys Val Asp Val
        115                 120                 125

Asp Glu Cys Ala Thr Asp Ser His Gln Cys Asn Pro Thr Gln Ile Cys
    130                 135                 140

Ile Asn Thr Glu Gly Gly Tyr Thr Cys Ser Cys Thr Asp Gly Tyr Trp
145                 150                 155                 160

Leu Leu Glu Gly Gln Cys Leu Asp Ile Asp Glu Cys Arg Tyr Gly Tyr
                165                 170                 175

Cys Gln Gln Leu Cys Ala Asn Val Pro Gly Ser Tyr Ser Cys Thr Cys
            180                 185                 190

Asn Pro Gly Phe Thr Leu Asn Glu Asp Gly Arg Ser Cys Gln Asp Val
        195                 200                 205

Asn Glu Cys Ala Thr Glu Asn Pro Cys Val Gln Thr Cys Val Asn Thr
    210                 215                 220

Tyr Gly Ser Phe Ile Cys Arg Cys Asp Pro Gly Tyr Glu Leu Glu Glu
225                 230                 235                 240

Asp Gly Val His Cys Ser Asp Met Asp Glu Cys Ser Phe Ser Glu Phe
                245                 250                 255

Leu Cys Gln His Glu Cys Val Asn Gln Pro Gly Thr Tyr Phe Cys Ser
            260                 265                 270

Cys Pro Pro Gly Tyr Ile Leu Leu Asp Asp Asn Arg Ser Cys Gln Asp
        275                 280                 285

Ile Asn Glu Cys Glu His Arg Asn His Thr Cys Asn Leu Gln Gln Thr
    290                 295                 300

Cys Tyr Asn Leu Gln Gly Gly Phe Lys Cys Ile Asp Pro Ile Arg Cys
305                 310                 315                 320

Glu Glu Pro Tyr Leu Arg Ile Ser Asp Asn Arg Cys Met Cys Pro Ala
                325                 330                 335

Glu Asn Pro Gly Cys Arg Asp Gln Pro Phe Thr Ile Leu Tyr Arg Asp
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340 345 350

Met Asp Val Val Ser Gly Arg Ser Val Pro Ala Asp Ile Phe Gln Met
355 360 365

Gln Ala Thr Thr Arg Tyr Pro Gly Ala Tyr Tyr Ile Phe Gln Ile Lys
370 375 380

Ser Gly Asn Glu Gly Arg Glu Phe Tyr Met Arg Gln Thr Gly Pro Ile
385 390 395 400

Ser Ala Thr Leu Val Met Thr Arg Pro Ile Lys Gly Pro Arg Glu Ile
405 410 415

Gln Leu Asp Leu Glu Met Ile Thr Val Asn Thr Val Ile Asn Phe Arg
420 425 430

Gly Ser Ser Val Ile Arg Leu Arg Ile Tyr Val Ser Gln Tyr Pro Phe
435 440 445

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAAAAAAAAA AAAAAA 16

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAGCTTTCCC CANCTTGGGA GAAGGGCAGN ATTNAAAATT GGCTACCCCC ATTCANTTTC 60

CNCCTTGGCA NCCTGCTTTT NATTAATANC AAATTTATTC CNGGCCNCAA AAACCTNCNN 120

TAATTNCTTN GGTTTGCCAA ATCCGGNTNC NAACCCCCAA TNCAAAANGG CCTTCCACAA 180

TTGGGCCNTA AATTNCCCCC CNNACAAAAN TAAAACCCCG NNTGCTNAAC CAAATTCCAA 240

AAAAATNGCA AAACCTTTTA AACCCCCCCC AAATNCTATT AAACAAAAAA ATTTTGATCC 300

CCT 303

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1726 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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CAATCTGCAT TTTAATGATA ACAGAATTAA TTCTGGCCTC AAAAAGCTAC TATGATATCT 120

TAGGTGTGCC AAAATCGGCA TCAGAGCGCC AAATCAAGAA GGCCTTTCAC AAGTTGGCCA 180

TGAAGTACCA CCCTGACAAA AATAAGAGCC CAGATGCTGA AGCAAAATTC AGAGAGATTG 240

CAGAAGCATA TGAAACACTC TCAGATGCTA ATAGACGAAA AGAGTATGAT ACACTTGGAC 300

ACAGTGCTTT TACTAGTGGT AAAGGACAAA GAGGTAGTGG AAGTTCTTTT GAGCAGTCAT 360

TTAACTTCAA TTTTGATGAC TTATTTAAAG ACTTTGGCTT TTTTGGTCAA AACCAAAACA 420

CTGGATCCAA GAAGCGTTTT GAAAATCATT TCCAGACACG CCAGGATGGT GGTTCCAGTA 480

GACAAAGGCA TCATTTCCAA GAATTTTCTT TTGGAGGTGG ATTATTTGAT GACATGTTTG 540

AAGATATGGA GAAAATGTTT TCTTTTAGTG GTTTTGACTC TACCAATCAG CATACAGTAC 600

AGACTGAAAA TAGATTTCAT GGATCTAGCA AGCACTGCAG GACTGTCACT CAACGAAGAG 660

GAAATATGGT TACTACATAC ACTGACTGTT CAGGACAGTA GTTCTTATTC TATTCTCACT 720

AAATCCAACT GGTTGACTCT TCCTCATTAT CTTTGATGCT AAACAATTTT CTGTGAACTA 780

TTTTGACAAG TGCATGATTT CACTTTAAAC AATTTGATAT AGCTATTAAA TATATTTAAG 840

GGTTTTTTTT TTTGACAAAT TCAACATTCA ACGAGTAGAC AAAATGCTAA TTATTTCCCT 900

GATTAGGAAA GTTTCTTTAA AAAACACGTA ATTTTGCCTA GTGCTTTTTC TCTACCTGCC 960

CTTGGGCTCA CTAATATCAC CAGTATTATT ACCAAGAAAA TATTGAGTTT ACCTGATTAA 1020

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TCTTAGGTCT TAGTATGGAT TTATGTGTTT GTGTGTGTGT AGTTTATCCT CTCTCTCATC 1500

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CAACCTTACT ATGTGCAATA TTTTTAAATC CTGAGAAATG TGTGCTTTTG TTTTCGGATA 1620

GACTTATTTC TTTAGTTCTG CACTTTTCCA CATTATACTC CATATGAGTA TTAATCCTAT 1680

GGATACATAT TAAAACAAGT GTCTCATAAA AAAAAAAAAA AAAAAA 1726

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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1 5 10 15

Ile Thr Glu Leu Ile Leu Ala Ser Lys Ser Tyr Tyr Asp Ile Leu Gly
20 25 30

Val Pro Lys Ser Ala Ser Glu Arg Gln Ile Lys Lys Ala Phe His Lys
35 40 45

Leu Ala Met Lys Tyr His Pro Asp Lys Asn Lys Ser Pro Asp Ala Glu
50 55 60

```
Ala Lys Phe Arg Glu Ile Ala Glu Ala Tyr Glu Thr Leu Ser Asp Ala
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Asn Arg Arg Lys Glu Tyr Asp Thr Leu Gly His Ser Ala Phe Thr Ser
                85                  90                  95

Gly Lys Gly Gln Arg Gly Ser Gly Ser Ser Phe Glu Gln Ser Phe Asn
            100                 105                 110

Phe Asn Phe Asp Asp Leu Phe Lys Asp Phe Gly Phe Phe Gly Gln Asn
        115                 120                 125

Gln Asn Thr Gly Ser Lys Lys Arg Phe Glu Asn His Phe Gln Thr Arg
    130                 135                 140

Gln Asp Gly Gly Ser Ser Arg Gln Arg His His Phe Gln Glu Phe Ser
145                 150                 155                 160

Phe Gly Gly Gly Leu Phe Asp Asp Met Phe Glu Asp Met Glu Lys Met
                165                 170                 175

Phe Ser Phe Ser Gly Phe Asp Ser Thr Asn Gln His Thr Val Gln Thr
            180                 185                 190

Glu Asn Arg Phe His Gly Ser Ser Lys His Cys Arg Thr Val Thr Gln
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Arg Arg Gly Asn Met Val Thr Thr Tyr Thr Asp Cys Ser Gly Gln
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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1774 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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TAATGAAAAG CTGTTTGGCT TTTAAAAATG ATGCCACAGA AATCCTTTAT TCACATGTGG     240

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```

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GCTATGGAAC AAAGTACTGG AGCAGGAGGA GCTCCCAGGA GTGGCGGTGT GTCAATGACA 540

AAACCCGTAC CCAGAGAATC CAGCTGCAGT GCCAAGATGG CAGCACACGC ACCTACAAAA 600

TCACAGTAGT CACTGCCTGC AAGTGCAAGA GGTACACCCG GCAGCACAAC GAGTCCAGTC 660

ACAACTTTGA GAGCATGTCA CCTGCCAAGC CAGTCCAGCA TCACAGAGAG CGGAAAAGAG 720

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AGTAACCATC TGCTTTACAG ATTTGATTGC TTGGAAGACT CAAGCCTGCC ACTGCTGTTT 840

TCTCACTTGA AAGTATATGC TTTCTGCTTT GATCAAACCC AGCAAGCTGT CTTAAGTATC 900

AGGACCTTCT TTGGGAATAG TTTTTCCTTT TCAAGTTTTT CAAGATGTAG GTATATCCAT 960

GAATGCAATT TGCATTTAAA TTCCACGTAT CCTGTAGTTT TAATTCCTCA TTGTTCTTAA 1020

AAGACTGTTG ATACTATAAA CATCAGTGAA TCATTATATT TTAAAACAGA AAAGGGCTTC 1080

TCAGATACCC TCCATCTACT GGCCCATCCC CTCTCCTAAA CAAAACTCCT TCAAAACAGG 1140

TTAAAAAAAA TATGTTGTCA TGAATCTTCA CAGTAACATT TCAGAAAGGT GCTTTTTTGG 1200

TACTCTTCAT GGGAACAGTT TAGCAGCCAT GAGTGATCTT CCTTTGAAAG AGAATGAAAG 1260

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GATCTTTTTA AATCAATAAG ATGCTTTGTA AAGTTGAAAT AAGTAATACT TTCTTGTTTA 1680

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AACACTGCTA AAAGTTAAAA AAAAAAAAAA AAAA 1774

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
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Leu Met Lys Ser Cys Leu Ala Phe Lys Asn Asp Ala Thr Glu Ile Leu
            20                  25                  30

Tyr Ser His Val Val Lys Pro Val Pro Ala His Pro Ser Ser Asn Ser
        35                  40                  45

Thr Leu Asn Gln Ala Arg Asn Gly Gly Arg His Phe Ser Asn Thr Gly
    50                  55                  60

Leu Asp Arg Asn Thr Arg Val Gln Val Gly Cys Arg Glu Leu Arg Ser
65                  70                  75                  80

Thr Lys Tyr Ile Ser Asp Gly Gln Cys Thr Ser Ile Ser Pro Leu Lys
                85                  90                  95

Glu Leu Val Cys Ala Gly Glu Cys Leu Pro Leu Pro Val Leu Pro Asn
            100                 105                 110

Trp Ile Gly Gly Gly Tyr Gly Thr Lys Tyr Trp Ser Arg Arg Ser Ser
        115                 120                 125

Gln Glu Trp Arg Cys Val Asn Asp Lys Thr Arg Thr Gln Arg Ile Gln
    130                 135                 140

Leu Gln Cys Gln Asp Gly Ser Thr Arg Thr Tyr Lys Ile Thr Val Val
145                 150                 155                 160

Thr Ala Cys Lys Cys Lys Arg Tyr Thr Arg Gln His Asn Glu Ser Ser
                165                 170                 175

His Asn Phe Glu Ser Met Ser Pro Ala Lys Pro Val Gln His His Arg
            180                 185                 190

Glu Arg Lys Arg Ala Ser Lys Ser Ser Lys His Ser Met Ser
        195                 200                 205
```

What is claimed is:

1. A composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 6 to nucleotide 545;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK296 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK296 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

2. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

3. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 2.

4. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acid 128 to amino acid 153;

(c) fragments of the amino acid sequence of SEQ ID NO:2; and

(d) the amino acid sequence encoded by the cDNA insert of clone AK296 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins.

5. The composition of claim 4, wherein said protein comprises the amino acid sequence of SEQ ID NO:2.

6. The composition of claim 4, wherein said protein comprises the amino acid sequence of SEQ ID NO:2 from amino acid 128 to amino acid 153.

7. The composition of claim 2, further comprising a pharmaceutically acceptable carrier.

8. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 7.

9. A composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 136 to nucleotide 1473;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK533 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK533 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

10. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) the amino acid sequence of SEQ ID NO:4 from amino acid 58 to amino acid 73;

(c) fragments of the amino acid sequence of SEQ ID NO:4; and

(d) the amino acid sequence encoded by the cDNA insert of clone AK533 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins.

11. A composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 186 to nucleotide 1532;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 261 to nucleotide 1532;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AK647 deposited under accession number ATCC 98026;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AK647 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above; and

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

12. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 104;

(c) the amino acid sequence of SEQ ID NO:6 from amino acid 1 to amino acid 93;

(d) fragments of the amino acid sequence of SEQ ID NO:6; and

(e) the amino acid sequence encoded by the cDNA insert of clone AK647 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins.

13. A composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 30 to nucleotide 701;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 99 to nucleotide 701;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AM610 deposited under accession number ATCC 98026;

(e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

(f) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AM610 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

(h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

(j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above; and

(k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above.

14. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 96;

(c) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 81;

(d) fragments of the amino acid sequence of SEQ ID NO:10; and

(e) the amino acid sequence encoded by the cDNA insert of clone AM610 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins.

15. A composition comprising an isolated protein encoded by a polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 132 to nucleotide 752;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR260 deposited under accession number ATCC 98026;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR260 deposited under accession number ATCC 98026;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(f) above; and

(j) a polynucleotide which encodes a species homologue of the protein of (g) or (h) above.

16. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12;

(b) the amino acid sequence of SEQ ID NO:12 from amino acid 1 to amino acid 85;

(c) fragments of the amino acid sequence of SEQ ID NO:12; and

(d) the amino acid sequence encoded by the cDNA insert of clone AR260 deposited under accession number ATCC 98026;

the protein being substantially free from other mammalian proteins.

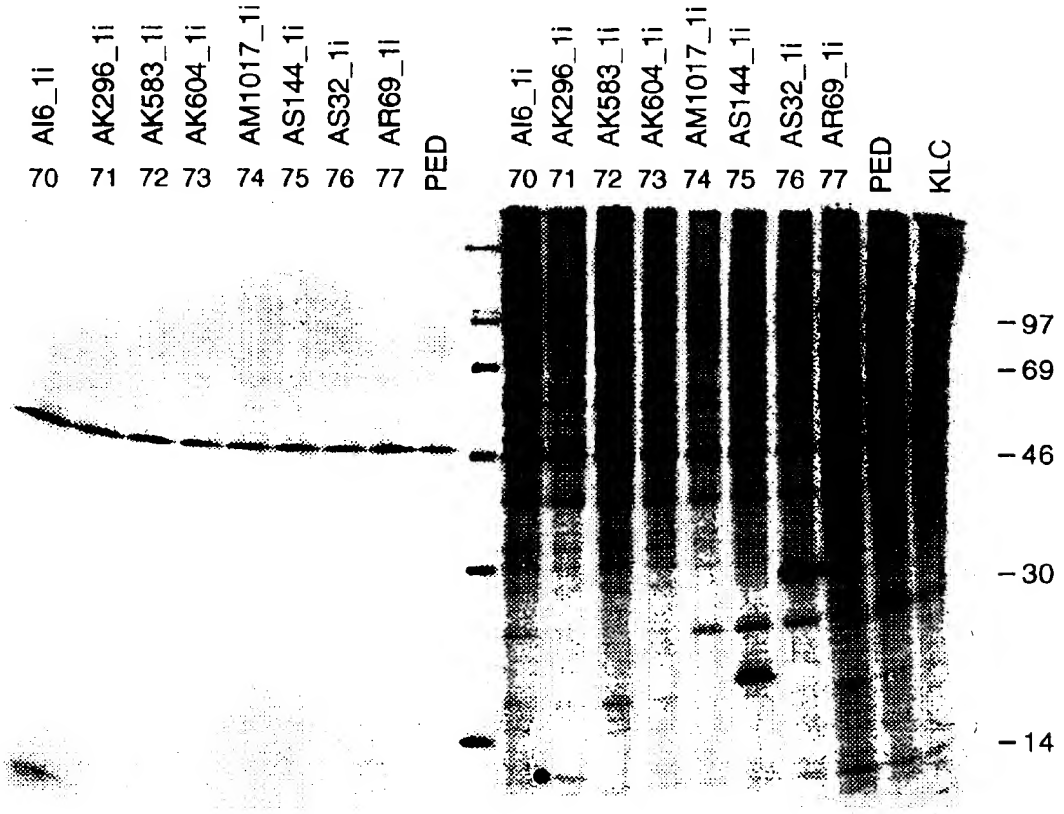
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Fig. 1

1/5

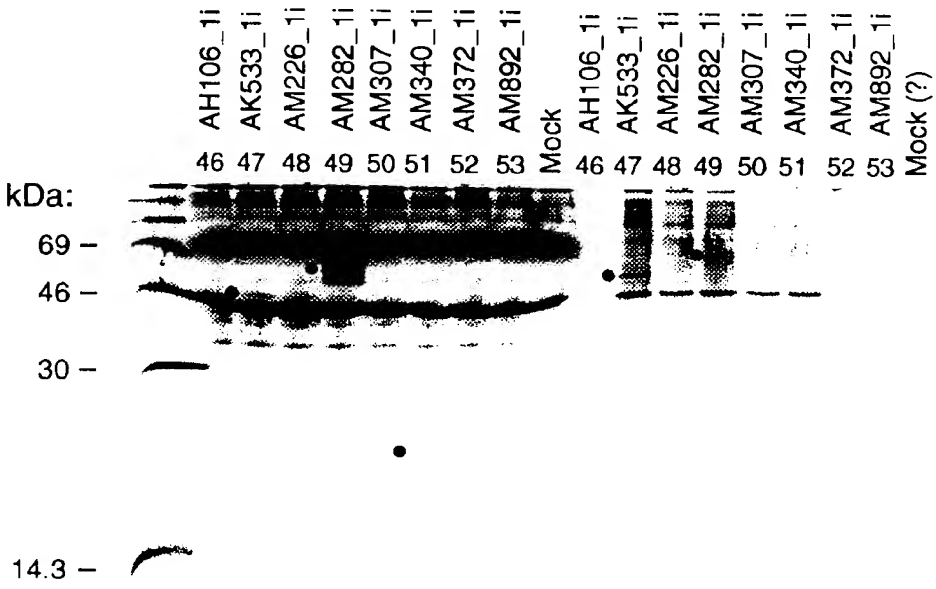
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Fig. 2

2/5

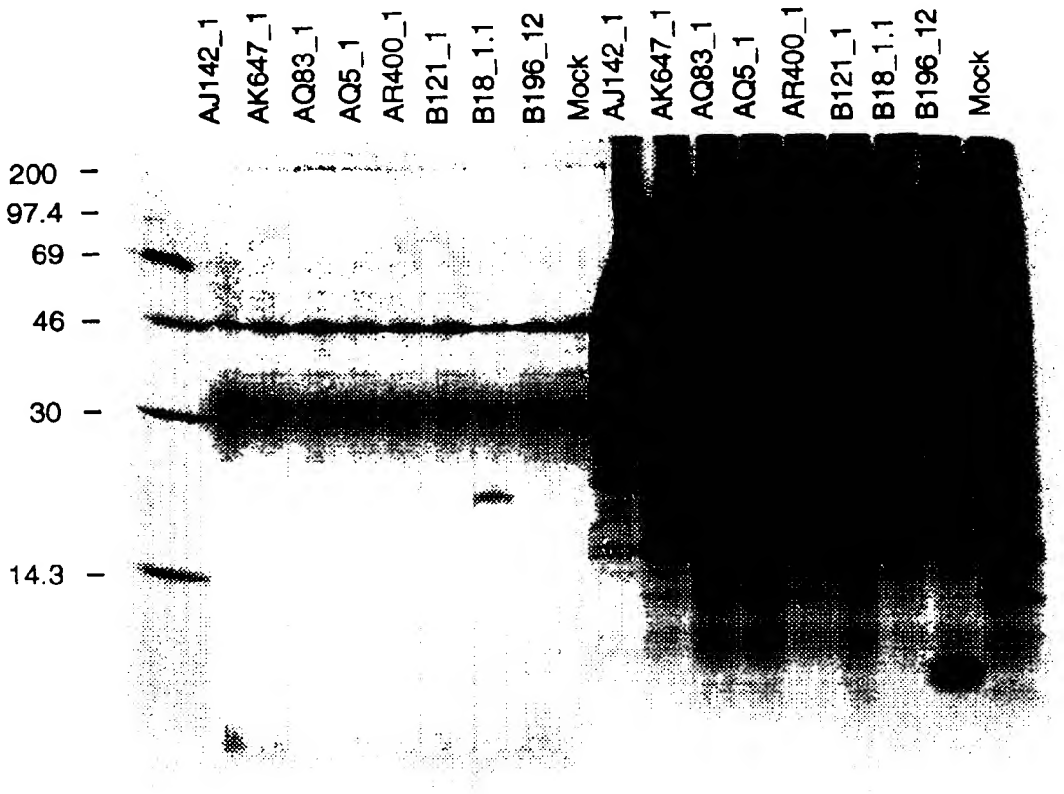
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Fig. 3

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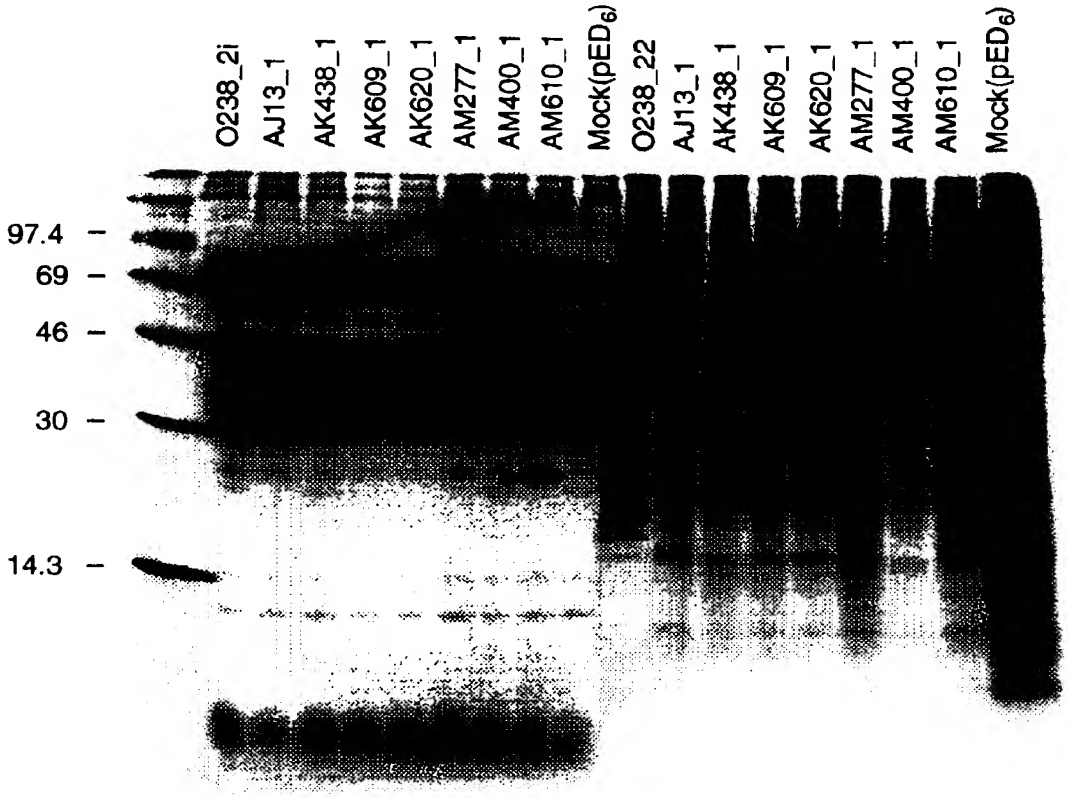
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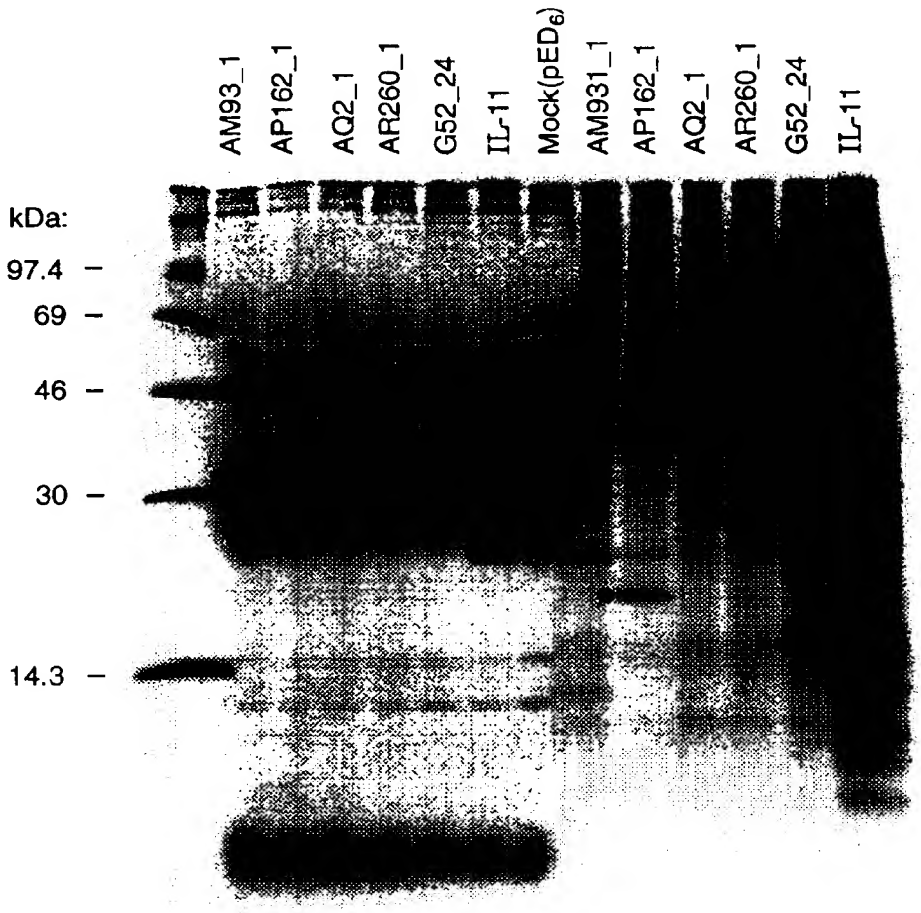
Fig. 4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97.4
69
46
30
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Fig. 5

5/5

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13530

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :C07K 2/00, 14/435; C12N 15/11, 15/12; A61K 38/02, 38/17
US CL :530/300, 350; 536/23.1, 23.5; 514/2, 12
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350; 536/23.1, 23.5; 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, GENBANK, MEDLINE
search terms: kenneth jacobs, T75226, AA171668, T2209, baculovirus, est, databas?, cDNA

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Database GenBank on STN. US National Library of Medicine (Bethesda, MD, USA). GenBank Accession Number T75226. yc86g12.rl Homo sapiens cDNA clone 22958 5'. 03 March 1995, see abstract. | 1-2, 4-7 |
| X | EP 0 606 734 A1 (FOLDES) 20 July 1994, especially Figure 1 and page 11. | 1-2, 4, 7 |
| Y | LENNON ET AL. The I.M.A.G.E. consortium: an integrated molecular analysis of genomes and their expression. Genomics. 01 April 1996. Vol. 33, pages 151-152, especially last paragraph of page 151 and first paragraph of page 152. | 1, 2, 4-7 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>17 SEPTEMBER 1998 | Date of mailing of the international search report<br>21OCT1998 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>CLAIRE M. KAUFMAN<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13530

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WAYE ET AL. Gene expression of adult human heart as revealed by random sequenceing of cDNA library. Protein Engineering. 1995. Vol. 8, page 90. | 1, 2, 4-7 |
| Y | THOMSEN ET AL. Applications of insect cell gene expression in pharmaceutical research. Bioprocess Technol. 1993, Vol. 17, (Insect Cell Culture Engineering), pages 105-138, especially sections 5.4 and 5.5. | 1, 2, 4-7 |
| Y | WO 94/01548 A2 (MEDICAL RESEARCH COUNCIL) 20 January 1994, especially page 10, line 38 and page 11, lines 9-10. | 1, 2, 4-7 |
| Y | BISHOP D.H.L. Membrane Protein Expression Systems. London:Portland Press. 1994, pages 83-124, especially, page 121, and last paragraph of page 99. | 1,2 4-7 |
| A | US 5,536,637 A (JACOBS) 16 July 1996, entire document. | 1-8 |
| A,P | US 5,712,116 A (JACOBS) 27 January 1998, entire document. | 1-8 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13530

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13530

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s), 1-8 drawn to an isolated protein encoded by or related to SEQ ID NO:1 or SEQ ID NO:2.
Group II, claim(s) 9-10, drawn to an isolated protein encoded by or related to SEQ ID NO:3 or SEQ ID NO:4.
Group III, claim(s) 11-12, drawn to an isolated protein encoded by or related to SEQ ID NO:5 or SEQ ID NO:6.
Group IV, claim(s), 13-14 drawn to an isolated protein encoded by or related to SEQ ID NO:9 or SEQ ID NO:10.
Group V, claim(s) 15-16, drawn to an isolated protein encoded by or related to SEQ ID NO:11 or SEQ ID NO:12.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The sequences encoding or related to each of the proteins claimed in Groups I-V are structurally unrelated, each to the other. Therefore, the inventions listed as Groups I-V do not relate to a single inventive concept.